EFFECT OF RATOON STUNTING DISEASE ON YIELD OF RECENTLY RELEASED SUGARCANE CULTIVARS IN LOUISIANA

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ABSTRACT

Four field experiments were conducted between 2000 and 2007 to determine the susceptibility of seven commercial sugarcane cultivars (HoCP 91-555, Ho 95-988, HoCP 96-540, L 97-128, L 99-226, L 99-233, and HoCP 00-950) released in Louisiana since 1999 to ratoon stunting disease (RSD) caused by the bacterium *Leifsonia xyli* subsp. *xyli*. Susceptibility was based on the percentage of vascular bundles in stalks colonized by the bacterium and yield loss of infected compared with uninfected plants. Cane and sucrose yields were determined in the plant-cane, first-ratoon, and second-ratoon crops of each experiment. Percent colonized vascular bundles (CVB) was determined for four randomly collected stalks from each plot. Percentages of CVB were high (>50%) in stalks of L 99-226 and L 99-233. Cane and sugar yields were reduced by 24% in the second-ratoon crop of L 99-226 and by an average of 32% across the plant-cane, first-ratoon, and second-ratoon crops of L 99-233. Moderately high percentages of CVB (15-50%) were observed in HoCP 91-555 and HoCP 00-950, but cane and sucrose yields were reduced only in the first-ratoon crop of HoCP 91-555. Percentages of CVB in stalks of the remaining three cultivars, Ho 95-988, HoCP 96-540, and L 97-128, were low (<15%), and no cane or sucrose loss was detected. Although no cane or sucrose yield loss was detected in cultivars with low percentages of CVB, and in only one of the two cultivars with moderate percentages of CVB, seed cane free of *L. xyli* subsp. *xyli* infection should be planted to avoid spread of the pathogen to more susceptible cultivars and to prevent the compounding of effects that may occur if the infected crop is exposed to additional biological or environmental stresses.

INTRODUCTION

Cultivar LCP 85-384 was released to the Louisiana sugarcane growers in 1993 (Milligan *et al.* 1994). Because of its superior yield potential compared with other available cultivars, the percent of the production area occupied by LCP 85-384 increased rapidly to a maximum of 91% in 2004 (Legendre and Gravois 2008). Concerned with declining yields of LCP 85-384 especially in later ratoon crops, growers are switching to more recently released cultivars (Legendre and Gravois 2008). From 1999 to 2007, seven cultivars were developed and released in Louisiana through the cooperative research of the Agricultural Research Service of the United States Department of Agriculture, the Louisiana Agricultural Experiment Station of Louisiana State University, and the American Sugar Cane League of the U.S.A, Inc.

Ratoon stunting disease (RSD) is caused by the systemic infection of the sugarcane vascular system by the bacterium *Leifsonia xyli* subsp. *xyli* (Grisham 2004). Cultivar LCP 85-

384 is moderately resistant to RSD based on the number of stalk vascular bundles colonized by the bacterium, yield loss observed in infected plants, and the extent of disease spread when compared with other commercial cultivars (Hoy *et al.* 2006). This study was conducted to determine the susceptibility of commercial cultivars released since 1999 based on comparing the number of colonized vascular bundles (CVB) in *L. xyli* subsp. *xyli*-infected plants and the yields of infected compared with bacteria-free plants.

MATERIALS AND METHODS

To establish diseased stock plants, a nursery was planted two years prior to each experiment with plots of each experimental cultivar. At the end of the first growing season, plants were inoculated with *L. xyli* subsp. *xyli* by first cutting the plants with a single-row, Cameco CH3500[1] chopper harvester (John Deere, Thibodaux, LA) with approximately 10 cm of the stalks remaining above the soil surface. Within a few days, the remaining above ground portions of the stalks were cut with a tractor-mounted, revolving disk cutting blade and the cut surface immediately sprayed with pathogen-contaminated cane juice extracted from diseased stalks. The inoculum was prepared by crushing stalks of a highly susceptible cultivar infected with *L. xyli* subsp. *xyli*. The juice was filtered through cheesecloth and diluted with distilled water (1:1; v:v). Presence of a high concentration of viable bacterial cells was verified by light microscopy. The next fall, a nursery was planted with two plots of each experimental cultivar. One plot was planted with stalks from the inoculated plants of the first nursery and was the source of *L. xyli* subsp. *xyli*-infected seed cane for the experiment, and the other plot was planted with stalks treated with hot water at 50 °C for 2 hr (Benda and Ricaud 1977) and was the source of uninfected seed cane. Seed cane from nurseries was examined for the presence of mosaic, leaf scald, and smut. These diseases were not observed in the nurseries, seed cane harvested from the nurseries, or in experimental plots.

Four field experiments were conducted between 2000 and 2007. The experiments were planted on 28 September 2000, 23 October 2001, 18 September 2003, and 10 September 2004, respectively. Each experiment consisted of three annually harvested crops: the plant-cane, first-ratoon, and second-ratoon crops. The crops of each experiment were treated as repeated measures on the same plots. Field experiments were arranged in a split-plot design with four replications. Main (treatment) plots had either plants infected with *L. xyli* subsp. *xyli* or uninfected plants, and the subplots were sugarcane cultivars. The plots in the first experiment were 6.1 m long and 5.3 m (3 rows) wide; in the second experiment, they were 6.1 m long and 3.6 m (two rows) wide; and in the third and fourth experiments, they were 7.6 m long and 5.3 m (three rows) wide.

Twelve to 18 cultivars were included in each experiment. Cultivar LCP 85-384 which occupied the highest percentage of the Louisiana sugarcane production area in 2000 (71%) (Legendre and Gravois 2007) was included in all four experiments (Table 1). Cultivar CP 70-321, released in 1978 and the second most widely grown cultivar at the beginning of these experiments (13%), was included in the first three experiments. By 2006, CP 70-321, occupied

[1] Mention of a trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product by the USDA-ARS and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

<1% of the Louisiana production area. At the time of planting each experiment, near-release cultivars in the final stages of testing (four or fewer years before release), were included in each experiment. As long as a cultivar was still under consideration for release or was released, it was included in subsequent tests. Consequently, the seven cultivars released from 1999 through 2007 (Table 1) were included in two to four experiments, initially as promising genotypes, then as released cultivars. Only data from released cultivars are presented in this report.

Standard plantation practices of cultivation, fertilization, and herbicide and insecticide application were used (Anonymous 1988). The experiments were planted on a Sharky clay soil at the United States Department of Agriculture, Agriculture Research Service, Sugarcane Research Laboratory research farm in Schriever, Louisiana. In the spring (mid-May to mid-June) following the fall planting, and in each spring of the ratoon crops, the number of shoots per plot was determined. In the fall (mid- to late-August), the total number of harvestable stalks (stalks that could be topped and cut by a mechanical harvester) in each plot was determined. The average percent stalk CVB by *L. xyli* subsp. *xyli* was determined for each cultivar with tissue blot enzyme immunoassay (TB-EIA) (Harrison 1988). Colonized vascular bundles and total vascular bundles were counted in a single core collected from the lowest internode of four randomly selected stalks harvested from each plot of all experiments in the fall of the plant cane crop. The percentage of CVB was determined for each core, and a mean was calculated for each treatment of each cultivar.

Each experiment was harvested without prior burning utilizing a single-row, chopper harvester, and the total weight of harvested cane in each plot was determined using a single-axle high-dump billet wagon containing three electronic load sensors (John Deere, Thibodaux, LA). The load sensors were mounted on the spindles at the ends of the axle and on the wagon's hitch where it was connected to the tractor. The 'weigh wagon' was also equipped with a billet sampler to obtain a random sample of sugarcane billets for sucrose quality analysis as the cane was being harvested. A sub-sample of the billeted cane was collected in this manner from each plot as it was harvested for juice and fiber analysis by the pre-breaker, core press method (Legendre 1992). Extracted juice was analyzed for Pol (percentage in juice), Brix (percentage soluble solids content in cane), sucrose (percentage in cane), and fiber (percentage in cane) and ultimately used to calculate theoretically recoverable sucrose (TRS) (kg Mg^{-1}). Plot weights and TRS were used to calculate cane (Mg ha^{-1}) and sucrose (kg ha^{-1}) yields.

Data were analyzed using SAS with PROC MIXED (SAS Institute 2001) with replication and experiment treated as random effects. Differences between treatment least square means were compared using the pdiff option (Saxton 1988) at the 0.05 probability level. Preliminary analysis of the data did not indicate a significant interaction between cane crop, cultivar, and treatment (RSD or control); however, the data appeared to show significant differences in the second ratoon crop. For this reason, the analysis was repeated using orthogonal contrasts to equally partition the error between crops. This analysis did confirm that significant differences were present between varieties in the second-ratoon crop; therefore, the data was discussed by crop.

RESULTS

The population of *L. xyli* subsp. *xyli* bacteria in the maturing stalks as indicated by percent CVB ranged from 4% in Ho 95-988 to 74% in L 99-233 (Table 1). Spring shoot populations were lower in *L. xyli* subsp. *xyli*-infected plants than in control plants of all crops of CP 70-321 and L 99-233 and in the plant-cane crop of L 97-128 (Table 2). Harvestable stalk populations

Table 1. Number of experiments in which each cultivar of nine Louisiana cultivars was included and mean percent colonized vascular bundles among stalks of *Leifsonia xyli* subsp. *xyli*-infected plants

Cultivar	Year of release	No. experiments	Percentage colonized vascular bundles	
Ho 95-988	2004	3	4	a[1]
HoCP 96-540	2003	4	8	a
L 97-128	2004	4	8	a
LCP 85-384	1993	4	29	b
HoCP 00-950	2007	2	34	bc
HoCP 91-555	1999	3	37	c
CP 70-321	1978	3	63	d
L 99-226	2006	3	71	de
L 99-233	2006	3	74	e

[1]Means within a column followed by the same letter are not statistically different using the F probability values and the PROC MIX macro as described by Saxton (1998) at alpha = 0.05.

Table 2. Spring shoot populations and fall harvestable stalk populations between control and *Leifsonia xyli* subsp. *xyli*-infected plants of nine Louisiana sugarcane cultivars

		Plant cane crop		First ratoon crop		Second ratoon crop	
Variable	Cultivar	Control	RSD	Control	RSD	Control	RSD
Spring shoots	CP 70-321	100	80 *[1]	88	52 *	74	33 *
($1000\ ha^{-1}$)	LCP 85-384	130	118	144	136	135	132
	HoCP 91-555	122	108	122	116	110	103
	Ho 95-988	119	115	135	133	139	152
	HoCP 96-540	117	111	125	128	122	134
	L 97-128	128	98 *	125	110	135	130
	L 99-226	107	96	108	101	108	82
	L 99-233	115	83 *	114	87 *	129	79 *
	HoCP 00-950	126	120	114	114	123	117
Harvestable stalks							
($1000\ ha^{-1}$)	CP 70-321	82	72	78	55 *	62	35 *
	LCP 85-384	104	108	120	121	98	106
	HoCP 91-555	103	100	106	102	92	90
	Ho 95-988	100	103	121	114	101	100
	HoCP 96-540	94	94	102	106	94	108 *
	L 97-128	94	84	99	94	95	94
	L 99-226	78	80	86	81	84	77
	L 99-233	108	95	108	95	112	94
	HoCP 00-950	105	101	110	99	91	87

[1] Difference between control and *Leifsonia xyli* subsp. *xyli*-infected plants was significant at P=0.05 (*) by Fischer's least significant difference (LSD) method.

were lower in infected plants than in control plants of first-ratoon and second-ratoon crops of CP 70-321 and higher in infected plants than in the control plants of the second-ratoon crop of HoCP 96-540 (Table 2).

Among the cultivars released since 1999, significant losses of cane and sucrose yields were observed in all three crops of L 99-233, in the second-ratoon crop of L 99-226, and the plant-cane crop of HoCP 91-555 (Table 3). Infection by L. *xyli* subsp. *xyli* resulted in higher cane yields of HoCP 96-540 in the second-ratoon crop. Theoretical recoverable sucrose (TRS) did not differ between uninfected and *L. xyli* subsp. *xyli*-infected plants, except that TRS was higher in infected plants than in control plants of the first-ratoon crop of L 97-128 (Table 3).

Table 3. Comparison of theoretical recoverable sugar (TRS) and cane and sugar yields between control and *Leifsonia xyli* subsp. *xyli*-infected plants of nine sugarcane cultivars grown in Louisiana

		Plant cane crop		First ratoon crop		Second ratoon crop	
	Cultivar	Control	RSD	Control	RSD	Control	RSD
Cane yield ($Mg\ ha^{-1}$)	CP 70-321	102	91 [1]	72	61	72	43 *
	LCP 85-384	98	99	97	85	88	84
	HoCP 91-555	99	94 *	90	75	80	82
	Ho 95-988	111	109	107	99	108	99
	HoCP 96-540	125	123	103	110	102	118 *
	L 97-128	109	98	89	92	97	109
	L 99-226	118	113	99	91	109	83 *
	L 99-233	108	77 *	92	65 *	123	78 *
	HoCP 00-950	106	97	95	79	98	96
Sucrose yield ($kg\ ha^{-1}$)	CP 70-321	9442	9166	6270	5018	6660	3563 *
	LCP 85-384	9317	9430	8540	7793	8133	7646
	HoCP 91-555	10410	10126 *	8401	6846	7344	6885
	Ho 95-988	11277	11106	10137	9468	10149	9452
	HoCP 96-540	11354	10832	9305	9106	9163	10684
	L 97-128	10147	9521	7813	8851	9108	10803
	L 99-226	10967	11079	9027	8533	10421	7777 *
	L 99-233	9363	7318 *	8046	5776 *	10718	7193 *
	HoCP 00-950	11180	9985	10123	8222	10253	9890
TRS ($kg\ Mg^{-1}$)	CP 70-321	92	101	88	82	92	81
	LCP 85-384	95	95	89	92	93	91
	HoCP 91-555	105	106	92	91	91	85
	Ho 95-988	101	102	94	96	94	95
	HoCP 96-540	94	93	88	90	90	91
	L 97-128	94	98	87	95 *	93	98
	L 99-226	93	98	90	94	95	94
	L 99-233	86	96	87	90	88	93
	HoCP 00-950	106	105	106	104	104	103

[1] Difference between control and *Leifsonia xyli* subsp. *xyli*-infected plants was significant at P=0.05 (*) by Fischer's least significant difference (LSD) method.

DISCUSSION

In a series of experiments conducted between 1978 and 1986, cane and sucrose yields were lower among the *L. xyli* subsp. *xyli*-infected plants of the six cultivars that occupied over 90% of the sugarcane production area in Louisiana at the time of the study (Grisham 1991). In contrast to the earlier study, only three of eight current commercial cultivars included in this study (HoCP 91-555, L 99-226, and L 99-233) showed yield loss among the *L. xyli* subsp. *xyli*-infected plants. Infected plants of cultivar CP 70-321 produced lower yields than the control plants in both the current and earlier studies (Grisham 1991). Because RSD is controlled in susceptible cultivars primarily by planting pathogen-free seed cane and practicing effective sanitation practices that reduce the mechanical spread of *L. xyli* subsp. *xyli* to healthy cane (Grisham 2004), resistance to RSD is not a selection criterion in the Louisiana breeding program. This study however indicates that resistance is present within the germplasm available to breeders.

There was good agreement between results for percent CVB and occurrence of significant yield loss among the cultivars released since 1999. The percentage of CVB was high(>50%) in stalks of L 99-226 and L 99-233, and cane and sucrose yields were reduced by 24% in the second-ratoon crop of L 99-226 (24%) and by an average of 32% across the plant-cane, first-ratoon, and second-ratoon crops of L 99-233. A moderately high percentage of CVB (15-50%) was observed in HoCP 91-555 and HoCP 00-950, but cane and sucrose yields were reduced only in the first-ratoon crop of HoCP 91-555. The percentage of CVB in stalks of the remaining three cultivars, Ho 95-988, HoCP 96-540, and L 97-128, was low (<15%) and no cane or sucrose loss was detected. In addition to indicating the potential for yield loss, percent CVB has also been positively correlated with the extent of *L. xyli* subsp. *xyli* spread by mechanical harvesters (Hoy and Grisham 2006).

The reduced population of shoots in the spring among plots of *L. xyli* subsp. *xyli*-infected plants of CP 70-321, L 99-233 and L 97-128 suggests that infected stubble (crowns) may be more susceptible than the uninfected plants to stresses during winter when the sugarcane plant is dormant. A reduction in the number of harvestable stalks, however, was observed only in infected plants of the first-ratoon and second-ratoon crops of CP 70-321. Since a reduction in harvestable stalk population was not detected among *L. xyli* subsp. *xyli*-infected plants of L 99-233, stalk weight was probably the more important component affecting cane yield loss among the infected plants. Theoretical recoverable sucrose generally did not differ between *L. xyli* subsp. *xyli*-infected and control plants among the cultivars included in this study. In other studies, *L. xyli* subsp. *xyli*-infected plants have had increased TRS resulting in less reduction in sucrose yield loss than cane yield due to RSD (M. P. Grisham, unpublished data).

Although no cane or sucrose yield loss was detected in cultivars with low percentages of CVB, and in only one of the two cultivars with moderate percentages of CVB, seed cane free of the *L. xyli* subsp. *xyli* infection should be planted to avoid spread of the pathogen to more susceptible cultivars and to prevent the compounding of effects that may occur if the infected crop is exposed to additional biological or environmental stresses. Greater yield reductions were reported among *L. xyli* subsp. *xyli*-infected plants subjected to moisture stress, either drought or water logging (Bailey and McFarlane 1999; Davis and Bailey 2000). It was demonstrated that a synergy can exist between *Sugarcane mosaic virus* [later reclassified as *Sorghum mosaic virus*

(SrMV) (Shukla *et al.* 1989)] and RSD resulting in greater losses than the additive effects when the two diseases occur independently (Koike 1974, 1982). Among the cultivars in this study that are currently being grown in Louisiana, L 99-226 and L 99-233 are moderately susceptible to SrMV. Recent studies have shown that all the cultivars in this study are susceptible to infection by *Sugarcane yellow leaf virus* (ScYLV), and infection of LCP 85-384, HoCP 96-540 and L 97-128 by ScYLV can cause yield loss (Grisham *et al.* 2009). No visual symptoms of yellow leaf caused by ScYLV were observed in this study. The stress of ratooning is believed to be greater in Louisiana and other temperate areas than in tropical and subtropical areas because of the exposure of the cane stubble to subfreezing temperatures. Two other stresses that Louisiana sugarcane may be exposed to are the application of growth regulating compounds to induce ripening and the deposition of crop residue following the harvest of unburned cane. Studies have been initiated to investigate the interaction of RSD and ScYLV and the effect of crop residue retention on *L. xyli* subsp. *xyli*-infected plants.

CONCLUSION

Among the seven sugarcane cultivars released in Louisiana between 1999 and 2007, only three were susceptible to yield loss caused by RSD caused by *L.* subsp. *xyli*; however, all were susceptible to infection by the bacterium. This is in contrast to the results of the earlier study conducted between 1978 and 1986 in which the six cultivars recommended for planting at that time were susceptible to yield loss caused by RSD. There was good agreement between the level of bacterial infection as determined by percent colonized vascular bundles and significant yield loss among the cultivars. Yield losses caused by ratoon stunting disease were demonstrated in two cultivars with the highest level of bacterial infection and in one with a moderate level of infection. Although no cane or sucrose yield loss was detected in four of the cultivars with low or moderate levels of infection, seed cane free of *L. xyli* subsp. *xyli* should be planted to avoid spread of the pathogen to more susceptible cultivars and to prevent the compounding of effects that may occur if the infected crop is exposed to additional biological or environmental stresses.

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